Evaluation of SSR allelic diversity on a broad sample of *Musa* species with emphasis on robustness of the results obtained

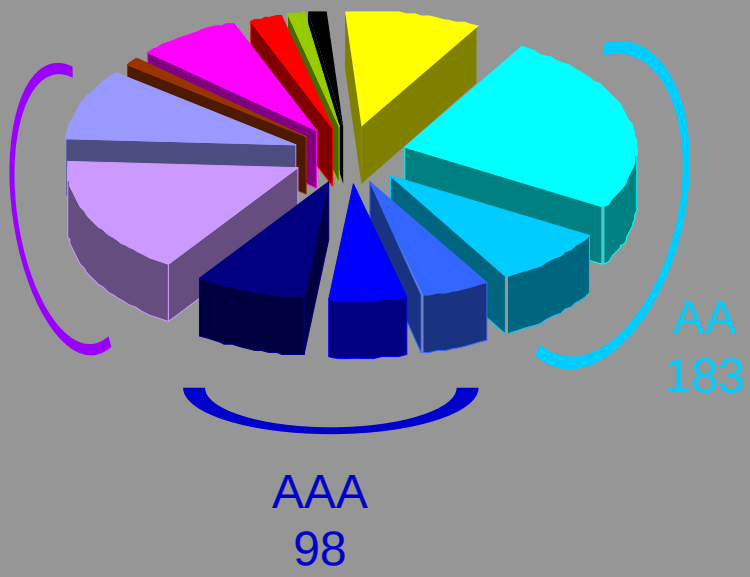
Initial frame of analysis

48 accessions supposed represented Musa genetic diversity genotyped in Cirad and IITA with 100 SSR

50 “best” SSR genotyped on world available diversity estimated to 1000 accessions in reference to *in vitro* collection of ITC

Background of analysis

Material



- Cv 139
- Wild 44
- mutika lujugira 26
- Cavendish 29
- Others AAA
- plantain 89
- Other 43
- AB 5
- ABB 40
- BB 12
- AS 7
- other sp 7
- Progeny or undefined 52

- **Cirad : 549 accessions coming from 3 main field collections**

  237 from Neufchateau (Cirad Guadeloupe)
  192 from IITA (Ibadan)
  120 from Carbap (Cameroun)

- **IITA : 511 accessions coming from same collections**

Markers

100 SSR ⟶ **1/3 stepwise 2/3 stepwise + indel** (25 IITA-25Cirad)

Cirad : 22 SSR a

SSR from AA Gobusik	range	Main all freq	Rare all*	Nb all
Ma1-32	43	0.53	7	20
Ma3-90	34	0.54	4	18
mMaCIR01	76	0.40	5	**22**
mMaCIR03	28	0.71	4	14
mMaCIR07	38	0.58	3	18
mMaCIR08	46	0.84	3	12
mMaCIR13	28	0.81	**0**	12
mMaCIR 24	60	0.85	9	19
mMaCIR27	28	0.68	4	12
mMaCIR39	40	0.78	7	20
mMaCIR40	38	0.62	8	17
mMaCIR45	22	0.83	1	9

SSR from BB PKW	range	Main all freq	Rare all*	Nb all.
mMaCIR150	13	0.84	0	**5**
mMaCIR152	**154**	0.41	1	12
mMaCIR164	154	0.37	7	17
mMaCIR195	56	0.55	**11**	21
mMaCIR196	26	0.71	3	12
mMaCIR214	20	0.69	2	6
mMaCIR 231	48	0.53	5	13
mMaCIR260	36	0.69	6	10
mMaCIR264	58	0.44	7	18
mMaCIR307	**12**	0.82	0	6
Mean			4	9

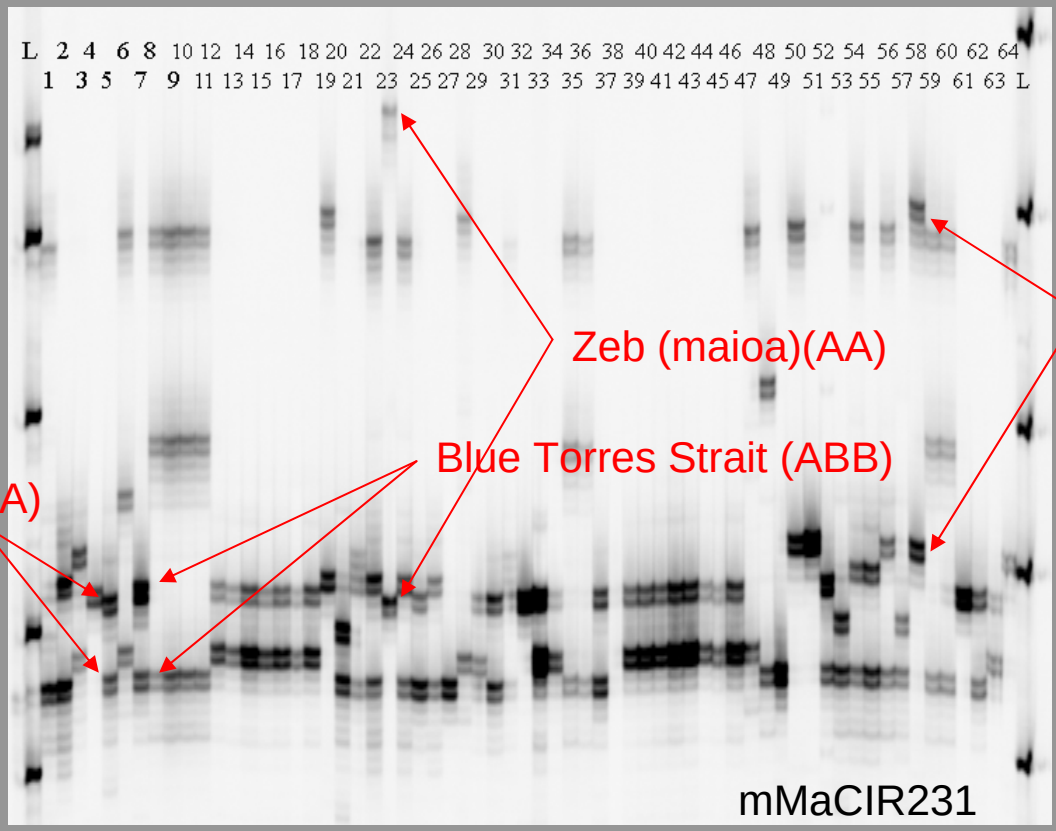
* Rare <1%

IITA : **26 SSR analyzed on a mean of 390 accessions (80 to 511) (capillary sequencer)**

Standards

Commercial ladder : gap in stepwise sizing
Internal standard mix of 3 accessions (Popoulou, Pisang Jari Buaya , Tomolo) : few amplifications
Accessions from Cavendish, Plantain and Mutika subgroups : available, distant,genetically conserved

Genotyping constraints : differences in intensity of allele's band



- Preferential amplification of short alleles
- Mutation(s) in annealing regions

Inability to asses doses to alleles then analysis to compare 2n and 3n
Difficulties to genotype

How to suppress differential or unspecific amplification ?

- Lowering level of scoring : not possible because some alleles are very little amplified, while they could give high signal in other accessions
- Suppressing alleles showing in some individuals weak intensity, but this leads to not genotyped accessions homozygous for the concerned alleles
- Increasing PCR stringency : to be tried but risk of loss of information and accuracy
- New SSR pannel

Analysis reliability : comparison of 4 common SSR between IITA and Cirad

		mMaCIR24		mMaCIR231		Ma1-32		Ma3-90	
Common accessions		45		284		250		246	
Nb Alleles : IITA Cirad		7 - 11		24 - 18		31 - 19		14 - 17	
Acces. allele nb> allele nb : IITA> Cirad		2	21%	30	9%	29	14%	20	8%
Cirad > IITA		12		24		30		23	
Allele nb > ploidy IITA Cirad		-		14		-		13 - 1	
Total alleles		64		557		434		513	
Alleles reliability		98%		92%		54% (235)		83% (425)	
Not reliable alleles(>2bp)		1		4 % (20)		7% (29)		9 % (49)	
+/-2bp		0		4% (27)		39% (170)		8 % (39)	
Alleles reliability of standard	Cavendish	94%		99%		11/25 46 %		29/43 (67%)	
	Plantain					10/23 43%		25/36 (69%)	
	Mutika					2/2		7/11 (63%)	
Differences of Alleles sizes		1bp		1 bp		Very variable		Variable	

Discriminating ability at the species level

Genome B specific alleles

	SSR	CIR 01 298	CIR 01 310	CIR 03 127	CIR 03 121	Ma 1-32 239
genome	Nb access.					
AA	186	-	-	-	-	-
AAA	100	-	-	-	-	3
AAB plantain	82	56	-	70	-	-
AAB	61	20	-	34	10	21
AB	9	2	-	2	5	7
ABB	37	12	21	20	24	30
BB	12	1/11	8/11	7/11	10/11	10/12

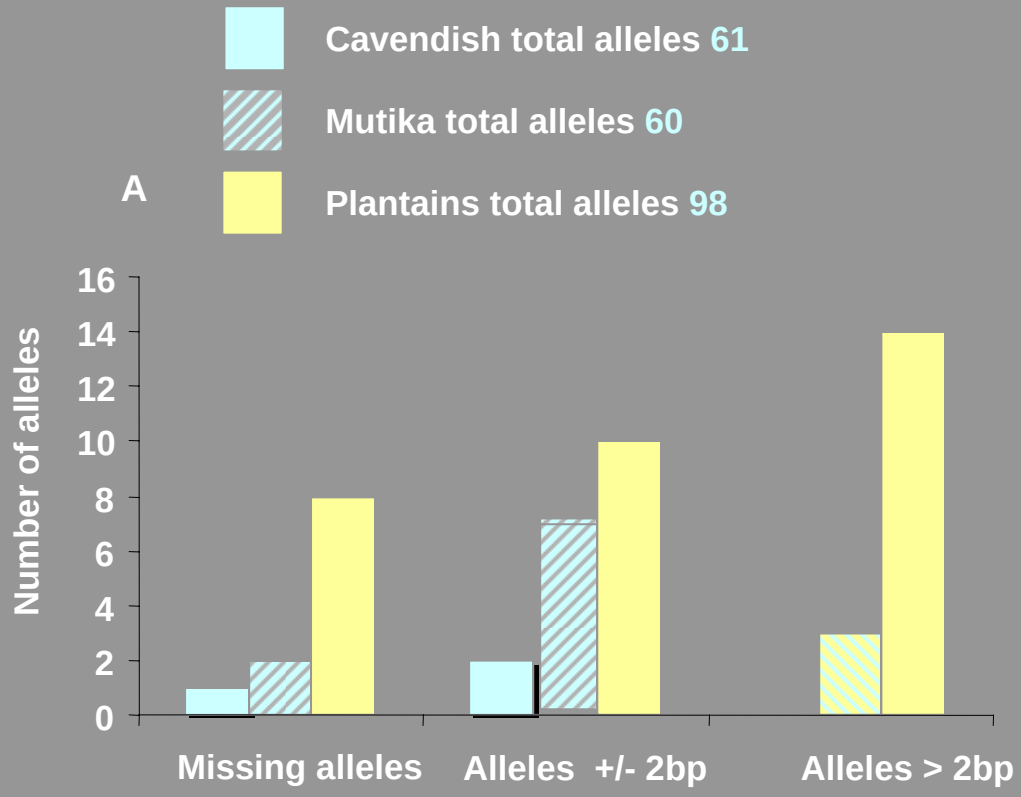
Likely lack of BB diversity

- Extinction of original BB ?
- Under sampling ? BB originated from south east Asia and are wide spread in this area

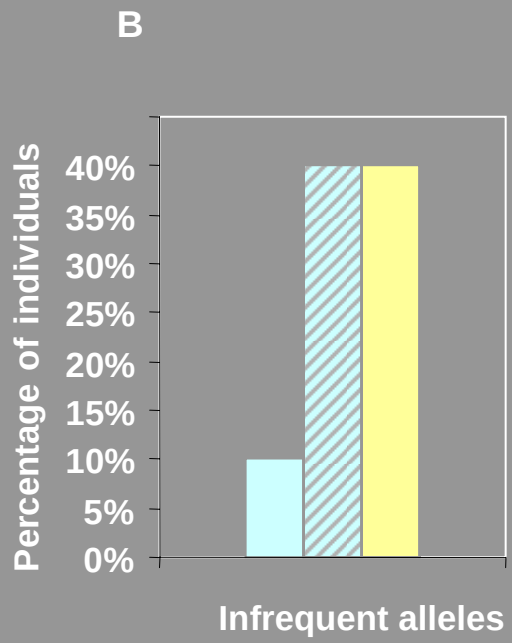
Collecting error or mislabelling of Foulah 4 (ABB) which is likely Foulah (Mutika)

Is subgrouping of AAB dessert banana into Pome, Nadan et Nendra padathti suitable ? (close neighbouring in diversity analysis, same triploid allelic combination, great homology in allelic pattern)

What is the status of Ambon group (phenotipically very closed to cavendish), very similar alleles to Cavendish but different pattern even between the 2 accessions



on <20% individuals



Percentage of individuals displaying different alleles from main allele(s) with a frequency under 20%.

Likely same parents for subgroups especially Cavendish but also Mutika and perhaps plantains

But originated from single sexual events ?
Likely for Cavendish. Very acceptable as an hypothesis for Mutika.
In this cas 2 events are not excluded for plantain

Are the origin of discrepancies coming from reading errors or somatic mutational events as Plantain and Mutika should have ? Both ?

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